



Radiographic and histological evaluations of the effects of meloxicam and flunixin meglumine on the repair of radial bone defects in a rabbit model

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ABSTRACT

The current study radiographically and histologically evaluated the effects of meloxicam and flunixin meglumine on the repair of radial bone defects in a rabbit model. Ninety New Zealand White rabbits (10-12 months, 1.5-2.6 kg) were randomly assigned into three groups. Following anesthesia, defects were created on the medial surface of the radius bone of the left forelimb with a diameter and depth of 3 mm. The animals were administered meloxicam, flunixin meglumine, and physiological serum (positive control) subcutaneously each day for 10 days. Hematoxylin and Eosin and Goldner's trichrome stainings, along with radiograph images were prepared to investigate the effects of the administered agents. The results did not show callus formation in bone defects on days 3 and 7. Defects were filled in meloxicam and positive control groups on day 14, while they were filled on day 21 in the flunixin meglumine group. On days 14 and 21, the meloxicam group outperformed the flunixin meglumine group in terms of callus formation, but it was higher in the flunixin meglumine group on day 28. It could be concluded that the administration of meloxicam is less effective in delaying the bone healing process.

Keywords

Flunixin meglumine, Histopathological, Meloxicam, Rabbit, Radiographic

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Abbreviations

NSAID: Non-steroidal anti-inflammatory drug
COX-1: Cyclooxygenase-1
COX-2: Cyclooxygenase-2
PGE2: Prostaglandins E2

Introduction

Bone fractures and segmental bone defects are major sources of patient morbidity and costs to the healthcare system [1]. Infection, tumor, trauma, surgery, and congenital aetiologies are major causes of bone defects [2]. The healing of bone defects is the main challenge for veterinarians and physical medicines [3]. Bone healing is a complex biological and biomechanical process and is characterized by three partially overlapping phases, namely the inflammatory phase, repair phase, and remodeling phase [4]. Inflammation plays a pivotal role in the bone healing process and immune-inflammatory modulation is a major challenge for treatment [5]. The NSAIDs, such as meloxicam and flunixin meglumine, are administered for the management of pain and inflammation in humans and animals. Meloxicam is a selective blocker of COX-2 that is used for managing rheumatoid arthritis, acute exacerbations of osteoarthritis, ankylosing spondylitis, skeletomuscular pains, and juvenile idiopathic arthritis [6]. It mostly exhibits its effects via COX-2 rather than COX-1 [7] and prevents COX-2 which results in inhibiting the conversion of arachidonic acid into pro-inflammatory prostaglandins [8]. Flunixin meglumine is another NSAID agent that is administrated to treat pain and signs of endotoxemia via the inhibition of COX isoenzymes [9]. It also reduces PGE2 concentrations in tissues [10]. Studies have investigated the effects of NSAIDs on the treatment of fractures and reported that NSAIDs have negative effects on mesenchymal stem cells due to the prevention of cell proliferation[11]. Another study showed that the administration of NSAIDs modulated the expression of osteogenic and chondrogenic marker genes [12]. Studies have also shown that NSAIDs may hinder bone healing and increase the risk of other complications [13]. Moreover, it was reported that NSAIDs did not change the proliferation and differentiation of osteoblasts but reduced the activity of plasminogen activators, metalloproteinases, and cathepsin B [14]. Regarding to the effects of NSAIDs on the bone healing process, the effects of meloxicam and flunixin meglumine on the bone healing process have not been still elucidated.

This study was conducted to compare and evaluate the effects of meloxicam and flunixin meglumine on the repair of radial bone defects in a rabbit model using H&E and Goldner's trichrome staining and radiograph images.

Results

Age and weight differences between the meloxicam, flunixin meglumine, and positive control groups were not found to be statistically significant ($p > 0.05$). All the rabbits underwent anesthesia and surgery and

survived until the end of the study.

Radiographic images

Figure 1 depicts the results of radiographic images. Our findings did not show significant differences on days 3 and 7. On the same days, no callus formation was observed. The filling of defects started on days 14 and 21 in the meloxicam and flunixin meglumine groups, respectively. Inflammation was similar between all the groups on the same days. Callus formation was significantly higher on day 28 compared to days 14 and 21 ($p < 0.05$). The findings indicated that the rabbits in the meloxicam group had more calli than those in the flunixin meglumine group on days 14 and 21. However, the score was significantly higher in the flunixin meglumine group on day 28 than in other groups. Furthermore, the scores were higher in the meloxicam group than in the flunixin meglumine group from day 28 until the end of study. There were significant differences between groups from day 42. Table 1 illustrates the scores for the bone healing process.

Histopathological results

Figure 2 shows histopathological results in different groups. The results of the first day in all groups revealed that the defect area was inflamed due to blood clot formation and increased growth of blood vessels. In the first week, blood clots were still visible in the defect area in all groups and collagen was gradually replacing blood clots. Blood clot and collagen ratios were 80% and 20% in the saline group, 85% and 15% in the flunixin meglumine group, and 95% and 5% in the meloxicam group, respectively. In the second week, clots were replaced with collagen and fibrotic tissue. The ratio of collagen to clot was 70% to 30%, 80% to 20%, and 90% to 10% in the positive control, flunixin meglumine, and meloxicam groups, respectively. In the third week, the clots were not seen in defects in control and meloxicam groups and they were also completely replaced with collagen. A small amount of clot (10%) was observed in the flunixin meglumine group. In the fourth week, the results showed that collagen was slightly replaced with cartilage in the control and flunixin meglumine groups, but no cartilage was observed in the meloxicam group. In the fifth week, the rabbits in the control groups had more cartilage. We observed that 30% of the samples had only collagen tissue, while collagen and cartilage tissue were equally apparent in 40% of the samples as the restoration process advanced. In addition, 30% of the samples had cartilaginous tissue, which was greater than their fibrous tissue. The difference between samples in NASID groups with collagen tissue and those who had progressed in their healing was 40%. In

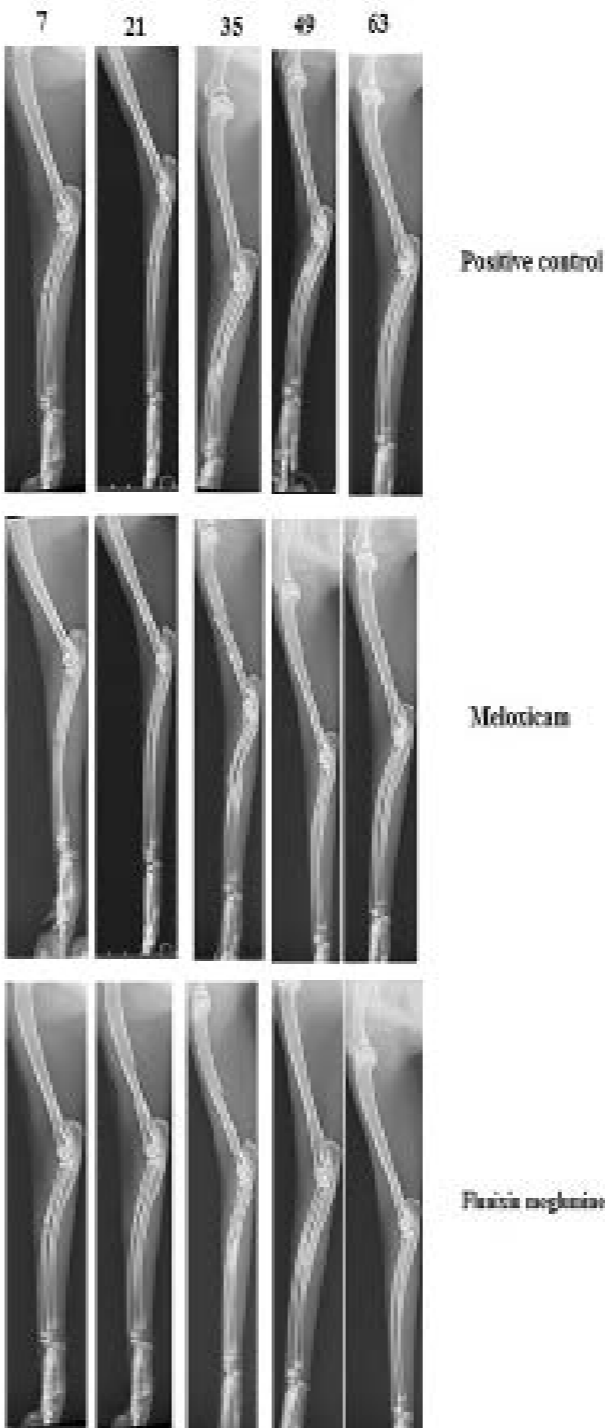


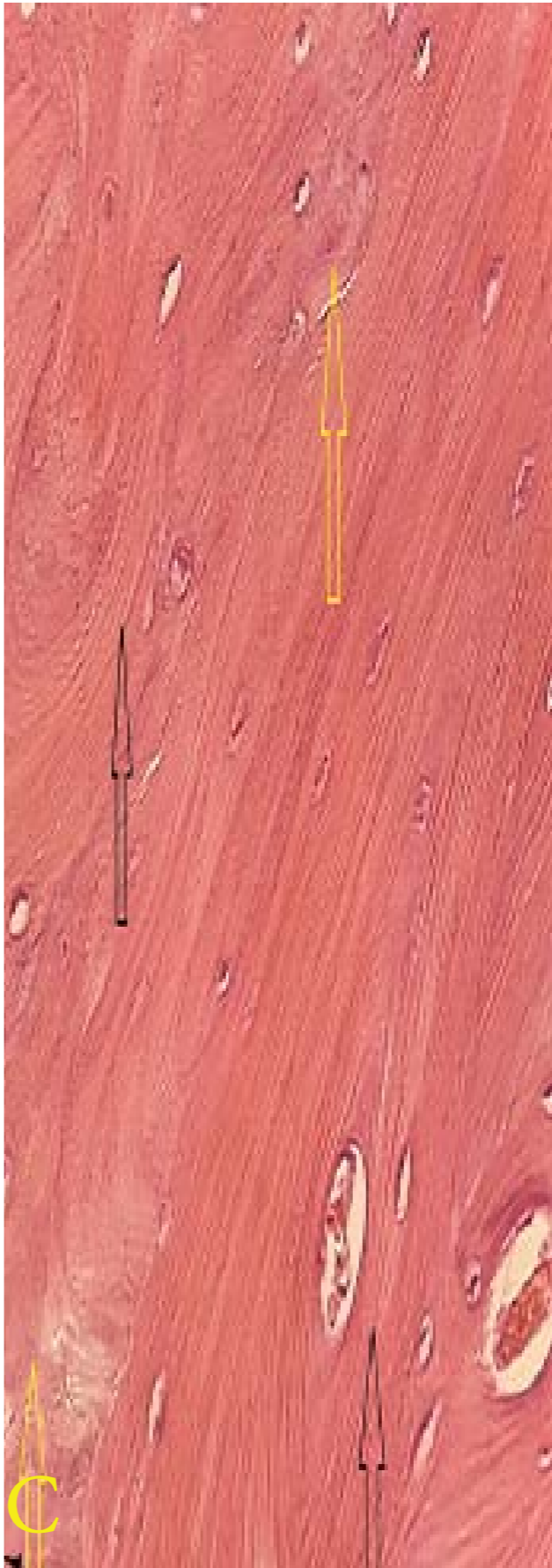
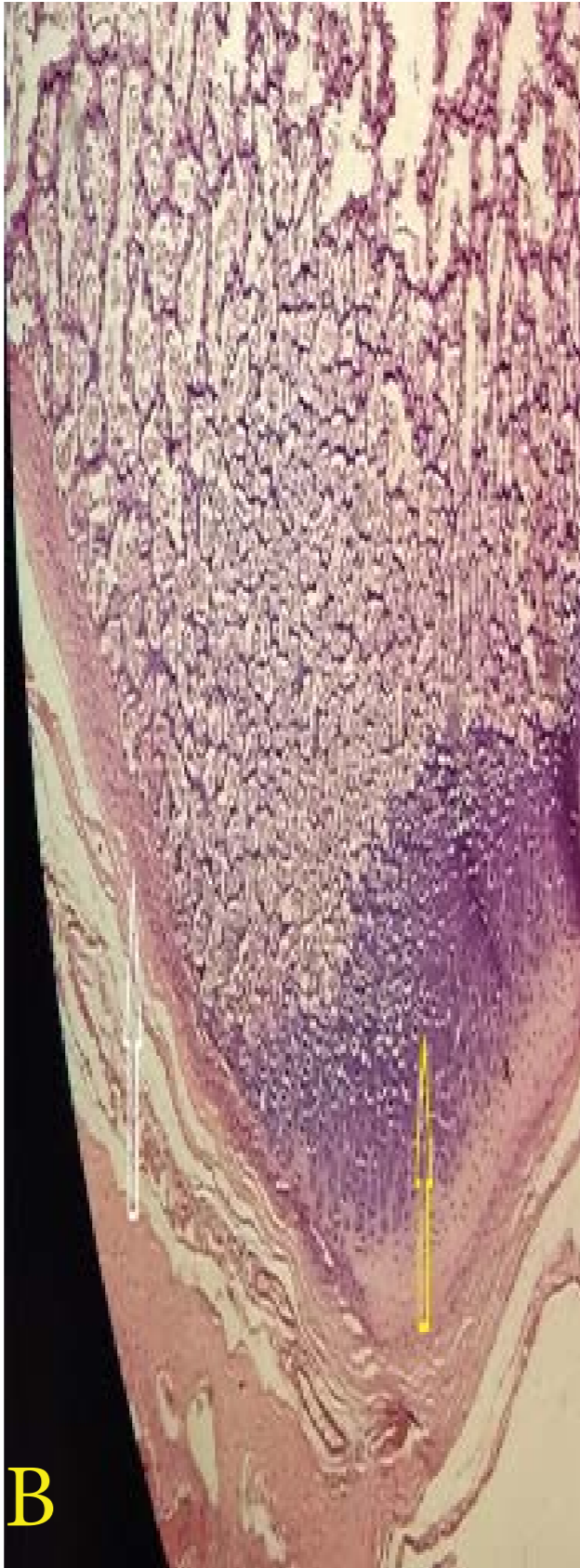
Figure 1. Radiograph images in different groups on days 7, 21, 35, 49, and 63

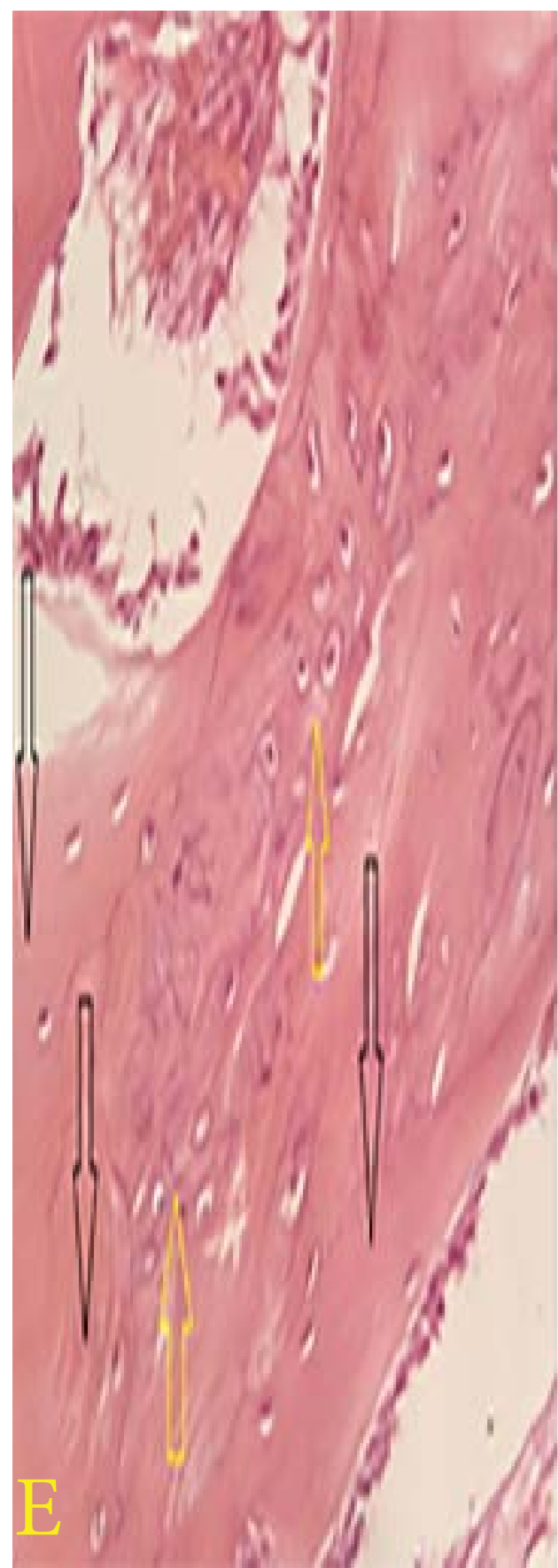
Table 1. The scores for the bone healing process

Groups	0	7	14	21	28	35	42	49	63
Control	0.00 ± 0.00	0.00 ± 0.00	0.85 ± 0.24	0.92 ± 0.18	1.42 ± 0.44	2.00 ± 0.00	2.57 ± 0.34	2.71 ± 0.26	2.64 ± 0.24
Flunixin M	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.64 ± 0.37	1.07 ± 0.18	1.14 ± 0.24	1.28 ± 0.26	1.42 ± 0.26	1.57 ± 0.34
Meloxicam	0.00 ± 0.00	0.00 ± 0.00	0.71 ± 0.39	0.78 ± 0.26	0.85 ± 0.24	1.28 ± 0.35	1.78 ± 0.26	1.88 ± 0.26	1.78 ± 0.26
P-values*	1.00	1.00	0.00	0.0231	0.012	0.0214	0.021	0.023	0.015

a.* Statistically significant ($p < 0.05$).

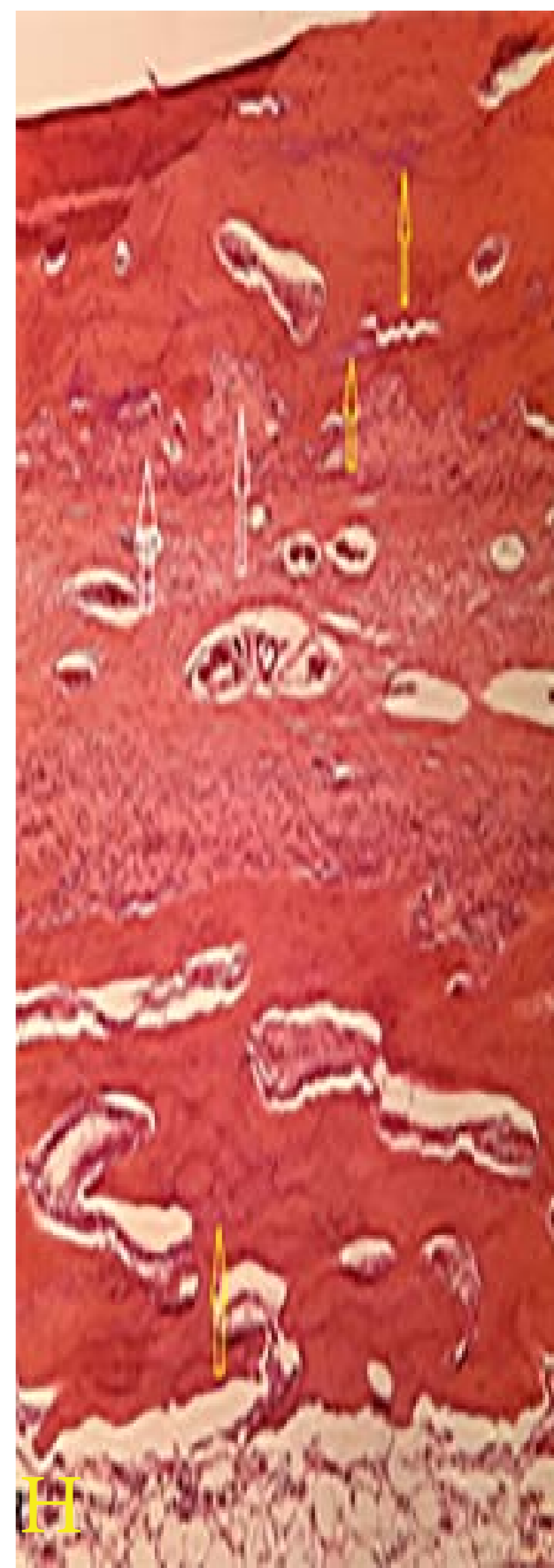
the 6th week, fibrotic tissue was completely replaced with cartilage tissue in some positive control samples (20%). In the NSAID groups, fibrotic tissue was completely replaced with cartilage tissue in some samples (10%) and cartilage tissue was significantly higher in the meloxicam group. In the 7th week, defects were significantly filled with cartilage in more samples in the control group and fibrotic tissue was completely replaced with cartilage tissue in some samples (10%) and cartilage tissue was significantly higher in the meloxicam group. The results of week 8 indicated that blood clots were not observed in the defect area in all groups and a significant amount of cartilage had replaced the clots. Consequently, there was more progress in the repair. The samples in which the amount of cartilaginous tissue was more than fibrous tissue were measured to be 20%. In addition to cartilaginous tissue, the samples also contained bone tissue, albeit in smaller amounts. In the flunixin meglumine group, samples were counted as having 30% more cartilaginous tissue than fibrous tissue. Moreover, in certain samples (40% of the subjects), the fibrotic tissue had vanished and the defects were cartilaginous. There was less bone tissue than cartilage tissue in 30% of the cases. Samples from the meloxicam group were measured to have 30% more cartilaginous tissue than fibrous tissue. In the 8th week's samples, it was noted that the defect area was completely cartilaginous, and 30% of the fibrotic tissue had vanished. In this eighth week, there were samples that contained bone tissue. However, the amount of bone tissue was low compared to cartilage tissue. These samples made up 40% of the total. The results of week 9 revealed that in 20% of the control group samples, there was more cartilaginous tissue than fibrous tissue, while the remaining samples had an equal quantity of cartilage and bone tissue. We observed that 20% of the samples in the flunixin meglumine group only had cartilage tissue, 40% of the samples had less bone tissue than cartilage tissue, and the remaining samples showed an equal distribution of cartilage and bone tissue. In the meloxicam group, bone tissue was found in 50% of the subjects in lower amounts than cartilage tissue. In addition, bone and cartilage tissue were found in similar amounts in the remaining samples.





E

F



G

H

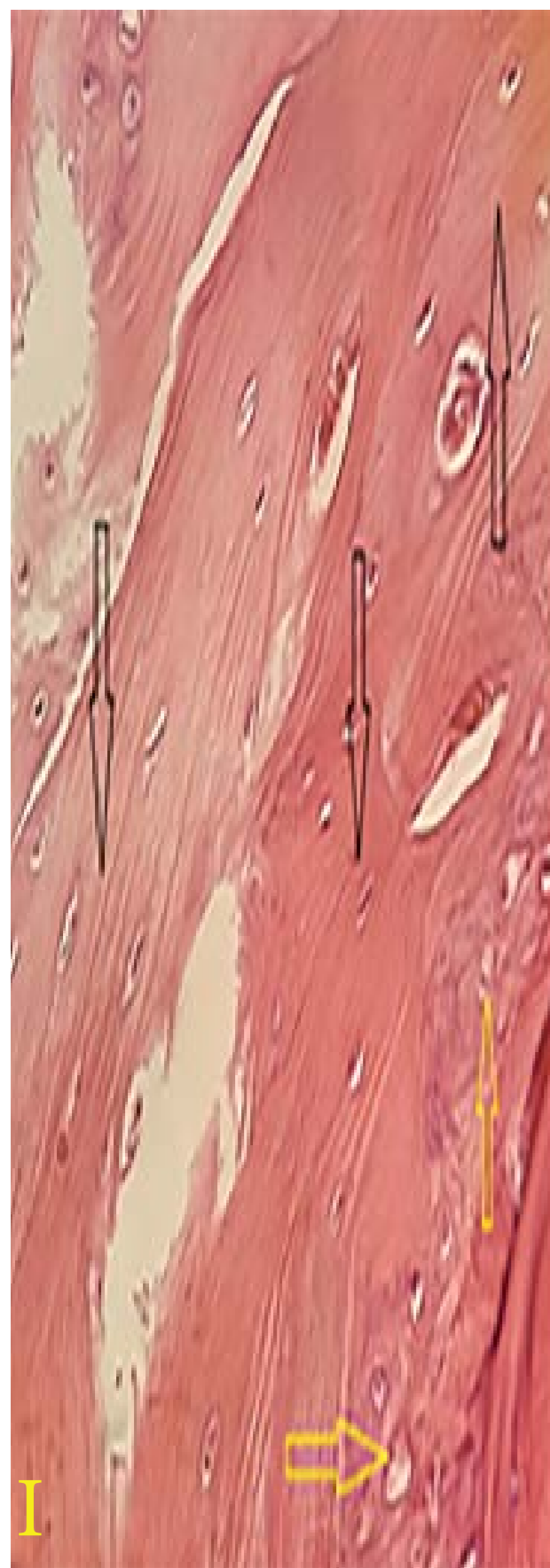


Figure 2.

A: Longitudinal section of the radius in rabbits in the third week and collagen is observable. Arrows show fibroblasts. B and C: Transversal section of the defect in the control group. B: There are similar ratios of fibrosis and cartilage tissues in the fifth week. Yellow and white arrows show cartilage and fibrotic tissues, respectively. C: Cartilage tissue is low and bone tissue is significantly found in the ninth week. Yellow and black arrows show cartilage and bone tissues. D-F: Transversal section of defects in the flunixin meglumine group. D: Fibrosis tissue is less and cartilage tissue is significantly observed in the seventh week. Yellow and white arrows show cartilage and fibrotic tissues, respectively. E & F: Cartilage tissue is much and bone tissue is slightly observed in the eighth and ninth weeks. Yellow and black arrows show cartilage and bone tissues, respectively. G-I: Transversal section of the defect in the meloxicam group. G: Fibrosis tissue (collagen) is observable in the fourth week. Arrows show fibroblasts. H: Cartilage tissue is much and fibrotic tissue is too low in the eighth week. I: There is a similar ratio of cartilage and bone in the ninth week. Yellow and black arrows show cartilage and bone tissues, respectively.

Discussion

This study aimed to evaluate radiographically and histologically the effects of meloxicam and flunixin meglumine on the repair of radial bone defects in a rabbit model. Results showed that NSAIDs significantly reduced the bone healing process compared to the control group. In the first week, the radiographic results did not show significant differences between groups ($p = 1$). On days 14 and 21, there were no significant differences between the meloxicam and the control groups. However, the bone healing process was significantly higher in the control group compared to other groups, indicating that meloxicam and flunixin meglumine did not cause any delay in the bone healing process in the first week compared to the control group. On the other hand, the administration of these agents delayed the bone healing process from the fourth week until the end of the study.

Our results are in line with previous studies that showed NSAIDs retard the bone healing process [11-14]. A delay in the bone healing process could be attributed to the effects of NSAIDs on PGE2 because bone and collagen formation is dependent on PGE2 [10]. Prostaglandins have pivotal roles in the regeneration of pericytes in injured bones and stimulate bone maturation. The results showed that the effects of meloxicam on the bone healing process were weaker compared with flunixin meglumine. It means that meloxicam has fewer adverse effects on the bone healing process due to its mechanism of action. Meloxicam primarily demonstrates its effects via inhibiting COX-2 [7] and preventing the conversion of arachidonic acid into pro-inflammatory prostaglandins [8], while flunixin meglumine exerts effects via inhibiting COX isoenzymes [10] and reducing PGE2 concentrations in tissues [14]. Flunixin meglumine appears to involve more COX isoenzymes than meloxicam and to have more potent effects. Histopathological results showed the presence of inflammation in the defects. According to the histopathological findings, the defects had inflammation which is a protective response to tissue trauma and/or a stimulus for removing harmful stimuli and starting the healing process [15]. It is argued that chronic sustained inflammation delays the healing process [16, 17]. The bone healing process might not be accompanied by inflammatory responses and may last for several months [18, 19]. Inflammation is the first step in bone healing and its prevention can expedite the healing process [16, 17]. The NSAIDs can reduce inflammation and accelerate the bone healing process. Our findings revealed that the administration of NSAIDs reduced inflammation. A reduction in inflammation may hasten the bone healing process, although NSAIDs inhibit it via other mechanisms. These agents inhibit the production

of PGE2 and bone regeneration via interaction with bone morphogenetic proteins [20, 21]. In addition to preventing DNA synthesis and reducing osteoblast proliferation, NSAIDs have no appreciable effects on bone mineralization [22]. Histopathological results indicated that the healing process slowed down in the second week and there was less fibrosis tissue formation in the NSAID groups than in the control group.

The results showed that the formation of cartilage and bone tissues was slower in the NSAID groups. Their actions on COX-2 may be responsible for the reduction in bone and cartilage production [6]. It can be stated that inflammation is essential for the bone healing process but it should rapidly occur. The NSAIDs inhibit inflammation and slow down bone healing. Our findings showed that NSAIDs inhibit the development of cartilage, collagen, and bone tissues and produce persistent clots over longer periods than in control groups.

It was concluded that NSAIDs inhibit callus formation in the defects. Histopathological findings confirmed that NSAIDs prevent callus formation, fibrosis, and bone tissue formation in defects. Flunixin meglumine had greater effects than meloxicam. It is suggested to administer the NSAIDs with lower side effects for decreasing pain during the bone fraction

Materials & Methods

Materials

Meloxicam (Boehringer-Ingelheim; Mobic, Germany), flunixin meglumine (Banamine injectable solution; Merck Animal Health, Madison, NJ, USA), ketamine (Alfasan; Woerden, Netherlands), and xylazine (Bayer; Leverkusen; Germany) were purchased.

Animals and surgery

Islamic Azad University, Urmia Branch approved all the procedures used in the current study for the care and treatment of animals (IR.IAU.URMIA.REC.1400.020). All the efforts were made to minimize pain. Ninety male New Zealand White rabbits (10-12 months, 1.5-2.6 kg) were prepared and kept in a room with free access to water and food. They were housed in a room at 24°C with a 12-h light/dark cycle. To induce anesthesia, each animal was intravenously administered with 2% pentobarbital sodium (1.5 ml/kg). Bone defects were created as reported by other researchers [23]. The animals were intramuscularly administered with 1 mg/kg of acepromazine and their left forelimbs were shaved from the middle of the arm to the end of the radius. To induce anesthesia, xylazine (8 mg/kg) and ketamine (70 mg/kg) were administered intramuscularly. Following the isolation of bone periosteum and soft tissues, a defect with a diameter and depth of 3 mm was created on the medial surface of the radius bone of the left forelimb of each rabbit. Soft tissue was overlapped on the defect and sutured. Cefazolin was intramuscularly administered for three days. The animals subcutaneously received 0.5 mg/kg meloxicam, 1 mg/kg flunixin meglumine, and physiological serum (positive control) 12 h/once for six days and then 24 h/once for four days.

Radiographic investigations

Radiographic evaluations were conducted on days 0, 7, 14, 21, 28, 35, 42, 49, and 63 and were scored as follows; 0: complete defect and lack of radiopacity in the defect region, 1: slight increase in radiopacity in the defect region and initiating the filling defects, 2: defect region not clear and an increase in radiopacity, and 3: defect completely filled and/or defect region similar to adjacent bones.

Histopathological evaluations

Rabbits were sedated with ketamine/xylazine and euthanized by intravenous injection of sodium pentobarbital (120 mg/kg) [24]. For histopathological investigation, sections were placed in formalin 10%, transferred to the histology lab, and cut. Next, the samples were dehydrated using degrading alcohol 70°C-100°C. They were placed in paraffin and frozen. The specimens were prepared at a diameter of 5 µm and deparaffinized and were stained with H&E and Goldner's trichrome. The sections were investigated by a light microscope.

Data analysis

The Shapiro-Wilk test was used to determine the normality of data distribution. Using SPSS software (version 21), the data were analyzed by the Mann-Whitney test. *P* < 0.05 was considered significant.

Authors' Contributions

Conceived and designed the experiments: HD, SS. Performed the experiments: SS. Analyzed the data: HD, SS, MMM, ARB. Research space and equipment: HD, MMM, ARB. Contributed reagents/materials/analysis tools: HD. wrote the paper: SS, HD.

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Conflict of interest

The authors declare that there is no conflict of interest.

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